Amendments to the Specification

With Markings

Page 1, lines 2 - 3, amend the cross-reference to related applications as follows:

This application ~~is a non-provisional of~~ claims the benefit of Danish patent application No. PA 2002 01098, filed July 13, 2002, and also claims the benefit under 35 USC 119(e) of US Provisional Patent Application No. 60/401,725, filed August 7, 2002~~, and claims the benefit of the priority thereof~~.

Page 1, paragraph spanning lines 5 – 9, amend to remove the period after tuberculoses as follows:

The present invention discloses a therapeutic vaccine against latent or active tuberculosis infection caused by the tuberculosis complex microorganisms (*Mycobacterium tuberculosis*[[.]], *M. bovis, M. africanum*). The invention furthermore discloses a multi-phase vaccine that can be administered either prophylactically or therapeutically as well as a diagnostic reagent for the detection of latent stages of tuberculosis.

Page 3, paragraph spanning lines 1-7, correct the reference to Turner, 2000, as follows:

This makes the development of a new and improved vaccine against TB an urgent matter, which has been given a very high priority by the WHO. Many attempts to define protective myco-bacterial substances have been made, and different investigators have reported increased resistance after experimental vaccination. However, these efforts have almost exclusively focused on the development of prophylactic vaccines for the prevention of disease (Doherty, 2002), and such vaccines have not been demonstrated to work if given in an immunotherapeutic fashion (J. Turner *et al., Infect and Immunity*, 2000, pp. 1706-1709).

Page 3, paragraph spanning lines 18 – 31, correct the reference to Turner, 2000 as follows:

As noted in the references cited above, it is already known that some genes are upregulated under conditions that mimic latency. However, these are a limited subset of the total gene expression during latent infection. Moreover, as one skilled in the art will readily appreciate, expression of a gene is not sufficient to make it a good vaccine candidate. The only way to determine if a protein is recognized by the immune system during latent infection with *M. tuberculosis* is to produce the given protein and test it in an appropriate assay as described herein. Of the more than 200 hundred antigens known to be expressed during primary infection, and tested as vaccines, less than a half dozen have demonstrated significant potential. So far only one antigen has been shown to have any potential as a therapeutic vaccine (Lowrie, 1999). However this vaccine only worked if given as a DNA vaccine, an experimental technique so far not approved for use in humans. Moreover, the technique has proved controversial, with other groups claiming that vaccination using this protocol induces either non-specific protection or even worsens disease (J. Turner *et al., Infect and Immunity,* 2000, pp. 1706-1709).

Page 41, line 22 – page 42, line 6, amend the paragraph as follows to remove the embedded hyperlink:

The term "sequence identity" indicates a quantitative measure of the degree of homology between two amino acid sequences of equal length or between two nucleotide sequences of equal length. The two sequences to be compared must be aligned to best possible fit allowing the insertion of gaps or alternatively, truncation at the ends of the protein sequences. The sequence identity can be calculated as $\frac{(N_{ref}-N_{dif})100}{N_{ref}}$, wherein N_{dif} is the total number of non-identical residues in the two sequences when aligned and wherein N_{ref} is the number of residues in one of the sequences. Hence, the DNA sequence AGTCAGTC [SEQ ID NO: 184] will have a sequence identity of 75% with the sequence AATCAATC, SEQ ID NO: 185 (N_{dif}=2 and N_{ref}=8). A gap is counted as non-identity of the specific residue(s), *i.e.* the DNA sequence AGTGTC [SEQ ID NO: 186] will have a sequence identity of 75% with the DNA sequence AGTCAGTC, SEQ ID NO: 187, (N_{dif}=2 and N_{ref}=8). Sequence identity can alternatively be calculated by the BLAST program *e.g.* the BLASTP program (Pearson, 1988 or ~~www.ncbi.nlm.nih.gov/cgi-bin/BLAST~~ on-line through the NIH web site). In one aspect of the invention, alignment is performed with the sequence alignment method ClustalW with default parameters as described by Thompson J., *et al* 1994 and as available ~~at http://www.ebi.ac.uk/clustalw/~~ through on-line sources.

Page 43, amend paragraph spanning lines 17-23 to remove "><" as follows:

Immunogenic portions of polypeptides may be recognized by a broad part (high frequency) or by a minor part (low frequency) of the genetically heterogeneous human population. In addition some immunogenic portions induce high immunological responses (dominant), whereas others induce lower, but still significant, responses (subdominant). High frequency~~><~~ or low frequency can be related to the immunogenic portion binding to widely distributed MHC molecules (HLA type) or even by multiple MHC molecules (Sinigaglia, 1988, Kilgus, 1991).

Page 55, amend the paragraph spanning lines 25 – 29 to remove italics and provide an initial capital on "Table" as follows:

~~*A number of M tuberculosis* genes are induced under low oxygen conditions. The up-regulation of the genes listed in table 2 has been determined at either the mRNA (Sherman, 2001) or protein (Boon, 2001, Rosenkrands, 2002) level. The coding region of these selected antigens is amplified by PCR using the primer sets listed in table 3~~.

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Page 58, amend the paragraph under the table, lines 1-7 to italicize "*M. tuberculosis*" as follows:

PCR reactions were carried out using Platinum Tag DNA Polymerase (GIBCO BRL) in a 50 µl reaction volume containing 60 mM Tris-SO_4 (pH 8.9), 18 mM Ammonium Sulfate, 0.2 mM of each of the four nucleotides, 0.2 µM of each primer and 10 ng of ~~M. tuberculosis~~ *M. tuberculosis* H37Rv chromosomal DNA. The reaction mixtures were initially heated to 95° C for 5 min., followed by 35 cycles of: 95° C for 45 sec, 60° C for 45 sec and 72° C for 2 min. The amplification products were precipitated by PEG/$MgCl_2$, and dissolved in 50 µL TE buffer.

Page 59, amend the paragraph spanning lines 17 – 31 to italicize "*E. coli*", as follows:

Recombinant proteins were purified from transformed ~~E. coli~~ *E. coli* BL21-SI cells cultured in 900 mL LBON medium containing 100 μg/mL at 30° C until OD_{600} = 0.4-0.6. At this point 100 mL 3 M NaCl was added and 3 hours later bacteria were harvested by centrifugation. Bacteria pellets were resuspended in 20 mL bacterial protein extraction reagent (Pierce) incubated for 10 min. at room temperature and pelleted by centrifugation. Bacteria were lysed and their DNA digested by treating with lysozyme (0.1 mg/mL) and DNase I (2.5 μg/mL) at room temperature for 30 minutes, with gentle agitation. The recombinant protein forms inclusion bodies and can be pelleted by centrifugation at 27.000 x g for 15 min. Protein pellets were solubilized by adding 20 ml of sonication buffer (8 M urea, 50 mM Na_2HPO_4, 100 mM Tris-HCl, pH 8.0) and sonicating 5 x 30 sec pulses interrupted by a 30 sec pause. After another centrifugation at 27.000 x g for 15 min., supernatants were applied to 10 mL TALON columns (Clontech). The columns were washed with 50 mL sonication buffer. Bound proteins were eluted by lowering pH (8 M urea, 50 mM Na_2HPO_4, 100 mM Tris-HCl, pH 4.5). 5 mL fractions were collected and analyzed by SDS-PAGE. Fractions containing re-

Page 63, amend the paragraph spanning lines 19 -27, to italicize "*M. tuberculosis*" as follows:

The Rv0569 induced protection against reactivation was determined by enumeration of bacteria in spleen and lung 7 weeks after the last immunization. Figure 7 shows the bacterial load in the lung and the spleen of both Rv0569-vaccinated, ESAT6-vaccinated, BCG vaccinated and un-vaccinated latently infected mice. There is a clear reduction in the level of viable bacteria in both spleen and lungs of the Rv0569 vaccinated mice, whereas neither ESAT6 nor BCG are able to inhibit the growth of the ~~M. tuberculosis~~ *M. tuberculosis* bacteria when given as a vaccine during latent infection. That is, the induction of Rv0569 T cell responses can participate in keeping the latent infection in check.

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Clean Version of Replacement Paragraphs:

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